July 2007

AllPrep™ DNA/RNA Micro Handbook

For simultaneous purification of genomic DNA and total RNA from the same small sample, including

- animal and human cells (≤5 x 10^5)
- animal and human tissues (≤5 mg)
- microdissected cryosections

Sample & Assay Technologies

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Contents

Kit Contents 5

Storage 5

Quality Control 5

Product Use Limitations 6

Product Warranty and Satisfaction Guarantee 6

Technical Assistance 6

Safety Information 7

Introduction 8

Principle and procedure 9

Equipment and Reagents to Be Supplied by User 11

Important Notes 12

Determining the amount of starting material 12

Handling and storing starting material 14

Disrupting and homogenizing starting material 15

Carrier RNA 17

Limitations of small samples 18

Protocols

- **Simultaneous Purification of Genomic DNA and Total RNA from Animal and Human Cells** 19
- **Simultaneous Purification of Genomic DNA and Total RNA from Animal and Human Tissues** 27
- **Simultaneous Purification of Genomic DNA and Total RNA from Microdissected Cryosections** 35

Troubleshooting Guide 40

Appendix A: General Remarks on Handling RNA 44

Appendix B: Storage, Quantification, and Determination of Quality of RNA 46

Appendix C: Storage, Quantification, and Determination of Quality of Genomic DNA 49

Appendix D: Purification of Total RNA Containing Small RNAs from Cells 51

Appendix E: Acetone Precipitation of Protein from Lysates 53

Appendix F: Optional On-Column DNase Digestion Using the RNase-Free DNase Set 54

Ordering Information 56

Kit Contents

AllPrep DNA/RNA Micro Kit	**(50)**
Catalog no.	**80284**
Number of preps	**50**
AllPrep DNA Mini Spin Columns (uncolored) (each in a 2 ml Collection Tube)	50
RNeasy® MinElute® Spin Columns (pink) (each in a 2 ml Collection Tube)	50
Collection Tubes (1.5 ml)	100
Collection Tubes (2 ml)	100
Buffer RLT Plus*	45 ml
Buffer RW1*	45 ml
Buffer RPE† (concentrate)	11 ml
RNase-Free Water	10 ml
Buffer AW1*† (concentrate)	19 ml
Buffer AW2† (concentrate)	13 ml
Buffer EB	15 ml
Carrier RNA, poly-A	310 µg
Handbook	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 7 for safety information.

† Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Storage

The AllPrep DNA/RNA Micro Kit should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions. RNeasy MinElute spin columns should be stored at 2–8°C.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of AllPrep DNA/RNA Micro Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

The AllPrep DNA/RNA Micro Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the AllPrep DNA/RNA Micro Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffer AW1 contains guanidine hydrochloride, Buffer RLT Plus contains guanidine thiocyanate, and Buffer RW1 contains a small amount of guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to the components of the AllPrep DNA/RNA Micro Kit.

Buffer AW1

Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46

Buffer RLT Plus

Contains guanidine thiocyanate: harmful. Risk and safety phrases:* R20/21/22-32, S13-26-36-46

Buffer RW1

Contains ethanol: flammable. Risk phrase:* R10

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R10: Flammable; R20/21/22: Harmful by inhalation, in contact with skin and if swallowed; R22: Harmful if swallowed; R32: Contact with acids liberates very toxic gas; R36/38: Irritating to eyes and skin; S13: Keep away from food, drink and animal feedingstuffs; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S46: If swallowed, seek medical advice immediately and show the container or label.

Introduction

The AllPrep DNA/RNA Micro Kit is designed to purify genomic DNA and total RNA simultaneously from the same, precious sample. Lysate is first passed through an AllPrep DNA spin column to selectively isolate DNA and then through an RNeasy MinElute spin column to selectively isolate RNA. Pure DNA and RNA are purified from the entire sample, in contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately. The kit is compatible with small amounts of a wide range of cultured cells, sorted cells, tissues, and microdissected samples of animal and human origin.

The AllPrep DNA/RNA Micro Kit allows the parallel processing of multiple samples in less than 40 minutes. Methods involving the use of toxic substances such as phenol and/or chloroform, or time-consuming and tedious methods such as alcohol precipitation, are replaced by the AllPrep DNA/RNA procedure.

Genomic DNA purified with the AllPrep DNA/RNA procedure has an average length of 15–30 kb depending on homogenization conditions.* DNA of this length is particularly suitable for PCR, where complete denaturation of the template is important to achieve the highest amplification efficiency. The purified DNA is ready to use in any downstream application, including:

- PCR and real-time PCR
- Southern, dot, and slot blot analyses
- Comparative genome hybridization (CGH)
- Genotyping, SNP analysis

With the AllPrep DNA/RNA procedure, all RNA molecules longer than 200 nucleotides are purified. The procedure provides an enrichment for mRNA, since most RNAs <200 nucleotides (such as 5.8S rRNA, 5S rRNA, and tRNAs, which together comprise 15–20% of total RNA) are selectively excluded.† The purified RNA is ready to use in any downstream application, including:

- RT-PCR
- Quantitative, real-time RT-PCR‡
- Differential display
- cDNA synthesis

* For purification of high-molecular-weight DNA (up to 150 kb), we recommend using either QIAGEN Genomic-tips or Blood & Cell Culture DNA Kits. For details, visit www.qiagen.com/DNA .

† For purification of miRNA and other small RNAs from cells and tissues, we recommend using the miRNeasy Mini Kit. For details, visit www.qiagen.com/miRNA .

‡ Visit www.qiagen.com/geneXpression for information on standardized solutions for gene expression analysis from QIAGEN.

- Northern, dot, and slot blot analyses
- Primer extension
- Poly A^+ RNA selection
- RNase/S1 nuclease protection
- Microarrays

Optionally, the AllPrep DNA/RNA procedure for cells can be modified to allow the purification of total RNA containing small RNAs such as miRNA (see Appendix D, page 51). In addition, if processing cells and easy-to-lyse tissues, optional steps for the AllPrep DNA/RNA procedure allow the isolation of protein by acetone precipitation (see Appendix E, page 53). The protein is denatured and suitable for applications such as SDS-PAGE and western blotting.

Principle and procedure

The AllPrep DNA/RNA procedure integrates QIAGEN's patented technology for selective binding of double-stranded DNA with well-established RNeasy technology. Efficient purification of high-quality DNA and RNA is guaranteed, without the need for additional RNase and DNase digestions.*

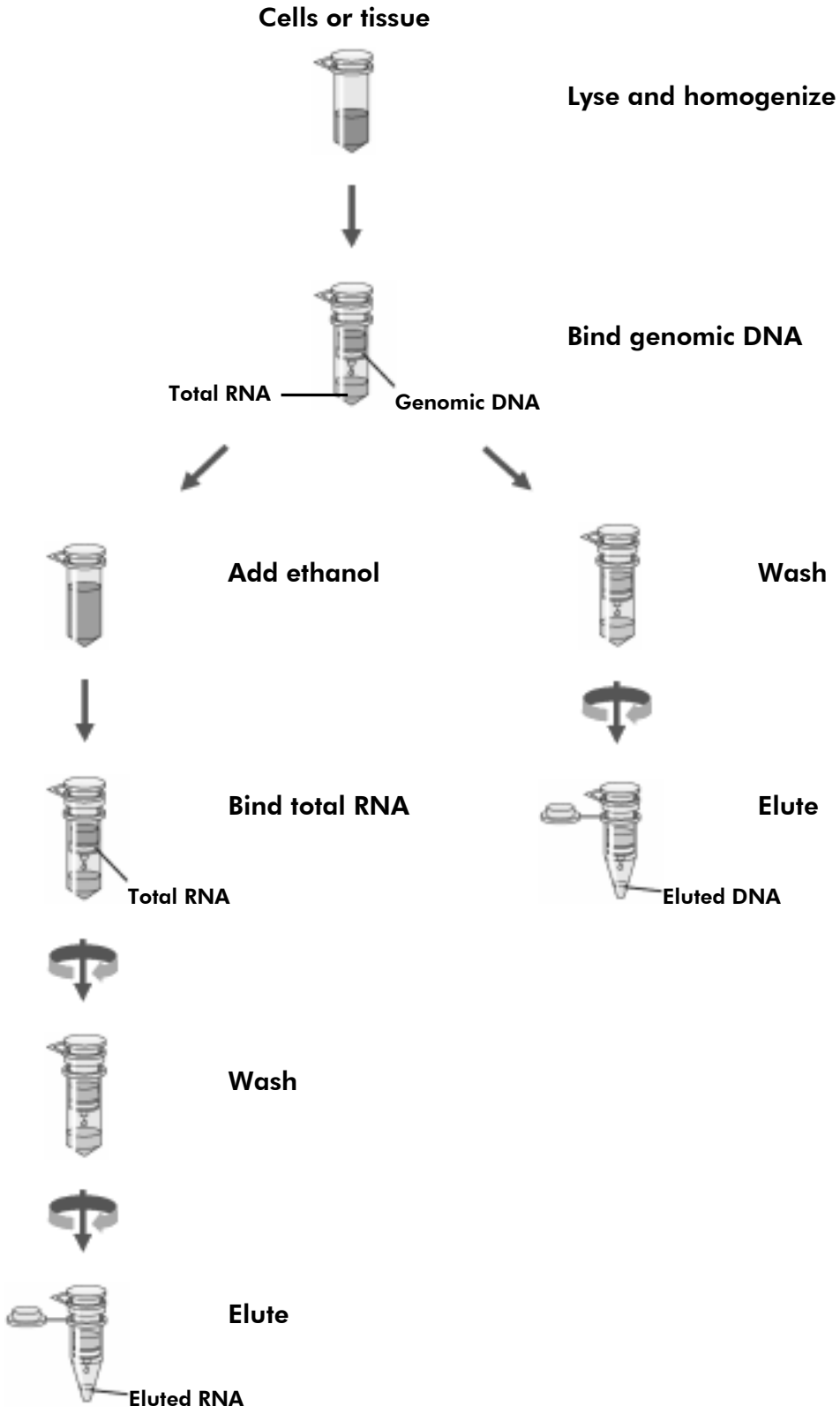
Biological samples are first lysed and homogenized in a highly denaturing guanidine-isothiocyanate–containing buffer, which immediately inactivates DNases and RNases to ensure isolation of intact DNA and RNA. The lysate is then passed through an AllPrep DNA spin column. This column, in combination with the high-salt buffer, selectively and efficiently binds genomic DNA. The column is washed and pure, ready-to-use DNA is then eluted.

Ethanol is added to the flow-through from the AllPrep DNA spin column to provide appropriate binding conditions for RNA, and the sample is then applied to an RNeasy MinElute spin column, where total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 14 µl water.

In this handbook, different protocols are provided for different starting materials. The protocols differ primarily in the lysis and homogenization of the sample. Once the sample is applied to the AllPrep DNA spin column, the protocols are similar (see flowchart, next page).

* Samples with particularly high DNA content may require additional DNase digestion.

AllPrep DNA/RNA Procedure



Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- 14.3 M β-mercaptoethanol (β-ME) (commercially available solutions are usually 14.3 M) or, alternatively, 2 M dithiothreitol (DTT) in water
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- 96–100% ethanol*
- 70% ethanol* in water
- Disposable gloves
- Reagent for RNA stabilization (see pages 14–15):
  - For cell samples: RNAprotect™ Cell Reagent† or liquid nitrogen
  - For tissue samples: RNA*later*® RNA Stabilization Reagent† (stabilizes RNA only), Allprotect™ Tissue Reagent† (stabilizes DNA, RNA, and protein), or liquid nitrogen
- Equipment for sample disruption and homogenization (see pages 15–17). Depending on the method chosen, one or more of the following are required:
  - Trypsin and PBS
  - QIAshredder homogenizer†
  - Blunt-ended needle and syringe
  - Mortar and pestle
  - TissueRuptor with TissueRuptor Disposable Probes†
  - TissueLyser†

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

† For ordering information, see page 56.

Important Notes

Determining the amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal nucleic acid yield and purity. The maximum amount that can be used is determined by:

- The type of sample and its DNA and RNA content
- The volume of Buffer RLT Plus required for efficient lysis
- The DNA binding capacity of the AllPrep DNA spin column
- The RNA binding capacity of the RNeasy MinElute spin column

When processing samples containing high amounts of DNA or RNA, less than the maximum amount of starting material shown in Table 1 should be used, so that the binding capacity of the spin columns is not exceeded.

When processing samples containing average or low amounts of DNA and RNA, the maximum amount of starting material shown in Table 1 can be used. However, even though the binding capacity of the spin columns is not reached, the maximum amount of starting material must not be exceeded. Otherwise, lysis will be incomplete and cellular debris may interfere with the binding of nucleic acids to the spin column membranes, resulting in lower yield and purity of DNA and RNA.

More information on using the correct amount of starting material is given in each protocol. Table 2 shows typical DNA and RNA yields from various cells and tissues.

Note: Although the AllPrep DNA spin column can bind a maximum of 100 µg DNA, the use of starting materials containing more than 20 µg DNA may lead to the purification of RNA containing small amounts of DNA. If the binding capacity of the RNeasy MinElute spin column is exceeded, RNA yields will not be consistent and less than expected. If lysis of the starting material is incomplete, DNA and RNA yields will be lower than expected, even if the binding capacity of the spin columns is not exceeded.

Table 1. Specifications of the spin columns in the AllPrep DNA/RNA Micro Kit

Specification	AllPrep DNA spin column	RNeasy MinElute spin column
Maximum binding capacity	100 µg DNA*	45 µg RNA
Maximum loading volume	700 µl	700 µl
Nucleic acid size distribution	DNA of 15–30 kb†	RNA >200 nucleotides‡
Minimum elution volume	30 µl	10 µl
Maximum amount of starting material		
■ Animal and human cells	5 x 10^5 cells	Entire flow-through from AllPrep DNA spin column
■ Animal and human tissues	5 mg	Entire flow-through from AllPrep DNA spin column

* Loading more than 20 µg DNA may lead to DNA contamination of the RNA eluate.

† Depending on homogenization conditions.

‡ Purification of total RNA containing small RNAs from cells is possible through a modification of the AllPrep DNA/RNA procedure. For details, see Appendix D, page 51.

Table 2. Typical yields of genomic DNA and total RNA with the AllPrep DNA/RNA Micro Kit

Sample type	Typical yield of genomic DNA (µg)	Typical yield of total RNA* (µg)
Cell cultures (5 x 10^5 cells)		
NIH/3T3	4	5
HeLa, Jurkat	3	8
COS-7	4	15
Mouse/rat tissues (5 mg)		
Brain	2–5	2–5
Heart	2–5	2–4
Kidney	7–15	10–15
Liver	7–15	20–30
Spleen	25–35	15–40
Thymus	25–50	20–40
Lung	5–10	5–10

* Amounts can vary due to factors such as species, developmental stage, and growth conditions. Since the AllPrep DNA/RNA procedure enriches for mRNA and other RNA species >200 nucleotides, the total RNA yield does not include 5S rRNA, tRNA, and other low-molecular-weight RNAs, which make up 15–20% of total cellular RNA.

Handling and storing starting material

Cells

After harvesting, cells should be immediately lysed in Buffer RLT Plus to prevent unwanted changes in the gene expression profile. This highly denaturing lysis buffer inactivates RNases and other proteins to prevent RNA degradation as well as downregulation or upregulation of transcripts.

If the cells are to be shipped to another lab for nucleic acid purification, they should be pelleted, frozen in liquid nitrogen, and transported on dry ice. Alternatively, the cells can be mixed with RNAprotect Cell Reagent at room temperature and then shipped at ambient temperature.

Tissues

RNA in harvested tissue is not protected until the sample is treated with RNA*later* RNA Stabilization Reagent, flash-frozen, or disrupted and homogenized in the presence of RNase-inhibiting or denaturing reagents. Otherwise, unwanted changes in the gene expression profile will occur. It is therefore important that tissue samples are immediately frozen in liquid nitrogen and stored at –70°C, or immediately immersed in RNA*later* RNA Stabilization Reagent. An alternative to RNA*later* RNA Stabilization Reagent is Allprotect Tissue Reagent, which provides immediate stabilization of DNA, RNA, and protein in tissue samples at room temperature.

The procedures for tissue harvesting and RNA protection should be carried out as quickly as possible. Frozen tissue samples should not be allowed to thaw during handling or weighing. After disruption and homogenization in Buffer RLT Plus (lysis buffer), samples can be stored at –70°C for months.

Disrupting and homogenizing starting material

Efficient disruption and homogenization of the starting material is an absolute requirement for all nucleic acid purification procedures. Disruption and homogenization are 2 distinct steps:

- **Disruption**: Complete disruption of plasma membranes of cells and organelles is absolutely required to release all the nucleic acids contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced nucleic acid yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the lysates produced by disruption. Homogenization shears high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of DNA and RNA and therefore significantly reduced yield and purity of nucleic acids. Excessive homogenization, on the other hand, results in shorter genomic DNA fragments.

Some disruption methods simultaneously homogenize the sample, while others require an additional homogenization step. Table 3 gives an overview of various disruption and homogenization methods, and is followed by a detailed description of each method.

Table 3. Disruption and homogenization methods

Sample	Disruption method	Homogenization method
Microdissected samples	Addition of lysis buffer	Vortexing
Cells and fine-needle aspirates (FNA)	Addition of lysis buffer	TissueRuptor or QIAshredder homogenizer or syringe and needle
Tissues	TissueRuptor*	TissueRuptor*
	TissueLyser†	TissueLyser†
	Mortar and pestle	QIAshredder homogenizer or syringe and needle

* Simultaneously disrupts and homogenizes individual samples.

† Simultaneously disrupts and homogenizes up to 192 samples in parallel. Results are comparable to those obtained using the TissueRuptor or other rotor–stator homogenizer.

Disruption and homogenization using the TissueRuptor

The TissueRuptor is a rotor–stator homogenizer that thoroughly disrupts and simultaneously homogenizes single tissue samples in the presence of lysis buffer in 15–90 seconds, depending on the toughness and size of the sample. The TissueRuptor can also be used to homogenize cell lysates. The blade of the TissueRuptor disposable probe rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. For guidelines on using the TissueRuptor, refer to the *TissueRuptor Handbook*. For other rotor–stator homogenizers, refer to suppliers' guidelines.

Note: Longer homogenization times with the TissueRuptor result in greater DNA fragmentation. Therefore, the homogenization time should be kept as short as possible if the DNA will be used in downstream applications that require long DNA fragments.

Disruption and homogenization using the TissueLyser

In bead-milling, tissues can be disrupted by rapid agitation in the presence of beads and lysis buffer. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the cells. The TissueLyser disrupts and homogenizes up to 48 tissue samples simultaneously when used in combination with the TissueLyser Adapter Set 2 x 24, which holds 48 x 2 ml microcentrifuge tubes containing stainless steel beads of 5 mm mean diameter. The TissueLyser can also disrupt and homogenize up to 192 tissue samples simultaneously when used in combination with the TissueLyser Adapter

Set 2 x 96, which holds 192 x 1.2 ml microtubes containing stainless steel beads of 5 mm mean diameter. For guidelines on using the TissueLyser, refer to the *TissueLyser Handbook*. For other bead mills, refer to suppliers' guidelines.

Note: Tungsten carbide beads react with Buffer RLT Plus and must not be used to disrupt and homogenize tissues.

Disruption using a mortar and pestle

For disruption using a mortar and pestle, freeze the tissue sample immediately in liquid nitrogen and grind to a fine powder under liquid nitrogen. Transfer the suspension (tissue powder and liquid nitrogen) into a liquid-nitrogen–cooled, appropriately sized tube and allow the liquid nitrogen to evaporate without allowing the sample to thaw. Add lysis buffer and continue as quickly as possible with the homogenization according to one of the 2 methods below.

Note: Grinding the sample using a mortar and pestle will disrupt the sample, but will not homogenize it. Homogenization must be performed afterwards.

Homogenization using QIAshredder homogenizers

Using QIAshredder homogenizers is a fast and efficient way to homogenize cell and tissue lysates without cross-contamination of samples. Up to 700 µl of lysate is loaded onto a QIAshredder spin column placed in a 2 ml collection tube, and spun for 2 minutes at maximum speed in a microcentrifuge. The lysate is homogenized as it passes through the spin column. QIAshredder homogenizers typically result in less DNA fragmentation compared with rotor–stator homogenizers.

Homogenization using a syringe and needle

Cell and tissue lysates can be homogenized using a syringe and needle. Lysate is passed through a 20-gauge (0.9 mm) needle attached to a sterile plastic syringe at least 5–10 times or until a homogeneous lysate is achieved. Increasing the volume of lysis buffer may be required to facilitate handling and minimize loss.

Carrier RNA

The AllPrep DNA/RNA Micro Kit contains poly-A RNA for use as carrier RNA. When added to lysates from very small samples, the carrier RNA may in some cases improve the recovery of total RNA. Carrier RNA is not required when processing more than 500 cells or more than about 2 µg tissue.

As demonstrated in many different RT-PCR systems, the small amounts of poly-A RNA used as carrier RNA in total RNA purification do not interfere with subsequent RT-PCR, even when oligo-dT is used as a primer for reverse transcription. Reverse-transcription reactions typically contain an excess of

oligo-dT primers, and the small amounts of poly-A used as carrier RNA are insignificant in comparison.

However, total RNA purified using poly-A RNA as carrier RNA is not compatible with protocols to amplify mRNA transcripts using oligo-dT primers. These include the Eberwine method and the QuantiTect® Whole Transcriptome Kit (the kit uses a mix of random and oligo-dT primers). For the Eberwine method, other types of RNA can be purchased separately for use as carrier RNA. Note, however, that tRNA and other RNAs <200 nucleotides will not bind to the RNeasy MinElute membrane and cannot be used as carrier RNA. For most applications, bacterial ribosomal RNA (e.g., from Roche, cat. no. 206938)* gives good results and can be used as an alternative to the poly-A RNA supplied with this kit. However, if amplifying mRNA transcripts with the QuantiTect Whole Transcriptome Kit, no carrier RNA of any type should be used in RNA purification.

Limitations of small samples

When purifying nucleic acids from particularly small samples (e.g., laser-microdissected samples), the amounts of DNA and RNA may be too small for quantification by spectrophotometry or even fluorometric assays. In this case, quantitative, real-time PCR or RT-PCR should be used for quantification.

When purifying DNA and RNA from less than 100 cells, stochastic problems with respect to copy number can occur. For example, if 20 cells are processed, and DNA is eluted in the recommended minimum volume of 30 µl, there will be less than 1 copy of each genomic DNA allele per microliter. Similarly, some RNA transcripts may be present at very low copy numbers per cell, or only in a fraction of all cells in the sample of interest.

Whole genome amplification or whole transcriptome amplification can be carried out to generate sufficient amounts of DNA or RNA if several downstream assays need to be performed from a single small sample. However, care should be taken to include a sufficient amount of starting material in the amplification reaction to avoid stochastic problems. REPLI-g® Kits and the QuantiTect Whole Transcriptome Kit provide highly uniform amplification of the genome and transcriptome, respectively. For details, visit www.qiagen.com/WGA and www.qiagen.com/goto/WTA .

* This is not a complete list of suppliers and does not include many important vendors of biological supplies. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA from Animal and Human Cells

Determining the correct amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal nucleic acid yield and purity. The maximum amount depends on:

- The RNA content of the cell type
- The DNA binding capacity of the AllPrep DNA spin column
- The RNA binding capacity of the RNeasy MinElute spin column (45 µg RNA)
- The volume of Buffer RLT Plus required for efficient lysis

In addition, cellular debris can reduce the binding capacity of the AllPrep DNA and RNeasy MinElute spin columns. If processing a cell type not listed in Table 2 (page 14) and if there is no information about its RNA content, we recommend starting with no more than 5 x 10^5 cells.

Do not overload the AllPrep DNA spin column, as this will lead to copurification of DNA with RNA. Do not overload the RNeasy MinElute spin column, as this will significantly reduce RNA yield and purity.

Counting cells is the most accurate way to quantitate the amount of starting material. As a guide, the number of HeLa cells obtained in various culture vessels after confluent growth is given in Table 4.

Important points before starting

- If using the AllPrep DNA/RNA Micro Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 44).
- If using the TissueRuptor, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- Cell pellets can be stored at –70°C for later use or used directly in the procedure. Determine the number of cells before freezing. Frozen cell pellets should be thawed slightly so that they can be dislodged by flicking the tube in step 2. Homogenized cell lysates from step 3 can be stored at –70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved. Avoid prolonged incubation, which may compromise RNA integrity. If any insoluble material is visible, centrifuge for 5 min at 3000–5000 x *g*. Transfer the supernatant to a new RNase-free glass or polypropylene tube, and continue with step 4.

Table 4. Growth area and number of HeLa cells in various culture vessels

Cell-culture vessel	Growth area (cm^3)*	Number of cells†
Multiwell plates		
96-well	0.32–0.6	4–5 x 10^4
48-well	1	1 x 10^5
24-well	2	2.5 x 10^5
12-well	4	5 x 10^5
6-well	9.5	1 x $10^{6‡}$
Dishes		
35 mm	8	1 x $10^{6‡}$
Flasks		
40–50 ml	25	3 x $10^{6‡}$

* Per well, if multiwell plates are used; varies slightly depending on the supplier.

† Cell numbers are given for HeLa cells (approximate length = 15 µm), assuming confluent growth. Cell numbers will vary for different kinds of animal and human cells, which vary in length from 10 to 30 µm.

‡ This number of cells exceeds the maximum binding capacity of the RNeasy MinElute spin columns. To process this many cells, split the lysate into appropriate aliquots (≤5 x 10^5 cells each) and load them onto separate gDNA Eliminator spin columns.

- Cells stored in RNAprotect Cell Reagent can also be used in the procedure. Transfer the entire sample, including any material deposited at the bottom of the storage vessel, to a centrifuge tube. Pellet the cells by centrifuging for 5 min at 5000 x *g*, and remove the supernatant by pipetting (if necessary, thaw the sample before centrifuging). Proceed immediately to step 2.
- Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- If purifying RNA from cell lines rich in RNases, we recommend adding β-mercaptoethanol (β-ME) to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh, or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- When processing <500 cells, carrier RNA may be added to the lysate before homogenization (see "Carrier RNA", page 17). Before using for the first time, dissolve the carrier RNA (310 µg) in 1 ml RNase-free water. Store this stock solution at –20°C, and use it to make fresh dilutions for each set of preps. The concentration of this stock solution is 310 µg/ml (i.e., 310 ng/µl). To make a working solution (4 ng/µl) for 10 preps, add 5 µl stock solution to 34 µl Buffer RLT Plus and mix by pipetting. Add 6 µl of this diluted solution to 54 µl Buffer RLT Plus to give a working solution of 4 ng/µl. Add 5 µl of this solution to the lysate in step 3. **Do not add the carrier RNA to the lysate if purifying RNA for use in oligo-dT–based amplification**.
- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Before using the kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) and 6 ml RNase-free water (supplied). The procedure also requires 70% ethanol, which can be prepared by diluting ethanol (96–100%) with distilled water (not supplied).
- Buffer RLT Plus may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. Harvest cells according to step 1a or 1b.

1a. Cells grown in suspension (do not use more than 5 x 10^5 cells): Determine the number of cells. Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g* in a centrifuge tube (not supplied). Carefully remove all supernatant by aspiration, and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

1b. Cells grown in a monolayer (do not use more than 5 x 10^5 cells): Cells grown in a monolayer in cell-culture vessels can be either lysed directly in the vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in a monolayer in cell-culture flasks should always be trypsinized.

To lyse cells directly:
Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

To trypsinize and collect cells:
Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x *g* for 5 min. Completely aspirate the supernatant, and proceed to step 2.

Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the conditions for nucleic acid purification. Both effects may reduce nucleic acid yields and purity.

2. Disrupt the cells by adding Buffer RLT Plus.

For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add 350 µl Buffer RLT Plus. Vortex or pipet to mix, and proceed to step 3.

If processing ≤1 x 10^5 cells, 75 µl Buffer RLT Plus can be added instead. This allows cell pelleting in smaller tubes. Pipet up and down to lyse the cells.

Note: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced nucleic acid yields.

For direct lysis of cells grown in a monolayer, add 350 µl Buffer RLT Plus to the cell-culture dish. Collect the lysate with a rubber policeman. Pipet the lysate into a microcentrifuge tube (not supplied). Vortex or pipet to mix, and ensure that no cell clumps are visible before proceeding to step 3.

If processing ≤1 x 10^5 cells, 75 µl Buffer RLT Plus can be added instead. This may be necessary for multiwell plates and cell-culture dishes. Pipet up and down to lyse the cells.

3. Homogenize the lysate according to step 3a, 3b, or 3c.

See "Disrupting and homogenizing starting material", page 15, for more details on homogenization. If processing ≤1 x 10^5 cells, they can be homogenized by vortexing for 1 min. After homogenization, proceed to step 4.

Note: If only 75 µl Buffer RLT Plus was used in step 2, transfer the lysate to a new 1.5 ml microcentrifuge tube, and adjust the volume to 350 µl with Buffer RLT Plus. Vortex for 1 min to homogenize and proceed to step 4.

Note: If processing <500 cells, 20 ng carrier RNA (5 µl of a 4 ng/µl solution) may be added to the lysate before homogenization. Prepare the carrier RNA as described in "Things to do before starting".

Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep and RNeasy MinElute spin columns. Homogenization with the TissueRuptor or QIAshredder homogenizer generally results in higher nucleic acid yields than with a syringe and needle.

3a. Pipet the lysate directly into a QIAshredder spin column (not supplied) placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Proceed to step 4.

3b. Place the tip of the TissueRuptor disposable probe into the lysate and operate the TissueRuptor at full speed until the lysate is homogenous (usually 30 s). Proceed to step 4.

Note: To avoid damage to the TissueRuptor and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

3c. Pass the lysate at least 5 times through a blunt 20-gauge needle (0.9 mm diameter) fitted to an RNase-free syringe. Proceed to step 4.

4. Transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm).

Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

5. **Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 13–16. Use the flow-through for RNA purification in steps 6–12.**

   If purification of total RNA containing small RNAs such as miRNA is desired, follow steps D1–D6 in Appendix D on page 51 instead of steps 6–12 in this protocol.

   Note: Do not store the AllPrep DNA spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA purification

6. **Add 1 volume (usually 350 µl) of 70% ethanol to the flow-through from step 5, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.**

   Note: The volume of 70% ethanol to add may be less than 350 µl if some lysate was lost during homogenization and DNA isolation.

   Note: When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

7. **Transfer the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.***

   Optional: If recovery of protein is desired, keep the flow-through on ice and follow steps E1–E5 in Appendix E on page 53.

   Reuse the collection tube in step 8.

8. **Add 700 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.***

   Reuse the collection tube in step 9.

   Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

9. **Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

   Reuse the collection tube in step 10.

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

10. **Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.**

    Prepare the 80% ethanol with ethanol (96–100%) and the RNase-free water supplied with the kit.

    Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

11. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.**

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

12. **Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**

    As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.

    The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

    For RT-PCR and real-time RT-PCR with the purified RNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit www.qiagen.com/PCR . For whole transcriptome amplification (WTA) of limited amounts of RNA, we recommend the QuantiTect Whole Transcriptome Kit. For details, visit www.qiagen.com/goto/WTA .

Genomic DNA purification

13. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 5. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (10,000 rpm) to wash the spin column membrane. Discard the flow-through.*

Reuse the spin column in step 14.

Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

14. Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and centrifuge for 2 min at full speed to wash the spin column membrane.

Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.

Note: After centrifugation, carefully remove the AllPrep DNA spin column from the collection tube. If the column contacts the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

15. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 50 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

16. Repeat step 15 to elute further DNA.

To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 15.

Note: To achieve a higher DNA concentration, elute with 2 x 30 µl Buffer EB. The final DNA yield, however, will be reduced.

For PCR and real-time PCR with the purified DNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit www.qiagen.com/PCR . QIAGEN kits and services for whole genome amplification (WGA) of limited amounts of DNA are also available. For details, visit www.qiagen.com/WGA .

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA from Animal and Human Tissues

This protocol is for the purification of genomic DNA and total RNA from easy-to-lyse animal and human tissues. For total RNA purification from frozen, microdissected tissue samples, see page 35.

Determining the correct amount of starting material

It is essential to use the correct amount of starting material in order to obtain optimal nucleic acid yield and purity. A maximum amount of 5 mg fresh or frozen tissue or 2–3 mg RNA*later* or Allprotect stabilized tissue (which is partially dehydrated) can generally be processed. For most tissues, the DNA binding capacity of the AllPrep DNA spin column, the RNA binding capacity of the RNeasy MinElute spin column, and the lysing capacity of Buffer RLT Plus will not be exceeded by these amounts. Typical DNA and RNA yields from various tissues are given in Table 2 (page 14).

For maximum RNA yields from liver, 50% ethanol (instead of 70% ethanol) should be used in step 5 of the procedure.

Some tissues, such as spleen and thymus, contain very high amounts of DNA, which may lead to copurification of RNA with trace amounts of DNA. For these tissues, we recommend performing DNase digestion on the RNeasy MinElute spin column membrane if the eluted RNA will be used in downstream applications sensitive to very small amounts of DNA (for further details, see Appendix F, page 54).

RNA yields from skeletal muscle, heart, and skin tissue may be low due to the abundance of contractile proteins, connective tissue, and collagen. For purification of genomic DNA and total RNA from these tissues, we recommend using the DNeasy® Blood & Tissue Kit and the RNeasy Fibrous Tissue Mini Kit, respectively (see page 56 for ordering information).

Do not overload the AllPrep DNA spin column, as this will lead to copurification of DNA with RNA. Do not overload the RNeasy MinElute spin column, as this will significantly reduce RNA yield and purity.

Weighing tissue is the most accurate way to quantitate the amount of starting material. As a guide, a 1.5 mm cube (3.4 mm^3) of most animal tissues weighs 3.5–4.5 mg.

Important points before starting

- If using the AllPrep DNA/RNA Micro Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 44).

- If using the TissueRuptor, ensure that you are familiar with operating it by referring to the *TissueRuptor User Manual* and *TissueRuptor Handbook*.
- If using the TissueLyser, ensure that you are familiar with operating it by referring to the operating instructions and *TissueLyser Handbook*.
- For optimal results, stabilize harvested tissues immediately in RNA*later* RNA Stabilization Reagent (see the *RNA*later *Handbook*) or Allprotect Tissue Reagent (see the *Allprotect Tissue Reagent Handbook*). Tissues can be stored in the reagent at 37°C for up to 1 day, at 15–25°C for up to 7 days, or at 2–8°C for up to 4 weeks (RNA*later*) or 6 months (Allprotect). Alternatively, tissues can be archived at –20°C or –80°C.
- Fresh, frozen, or RNA*later*/Allprotect stabilized tissues can be used. Tissues can be stored at –70°C for several months. Flash-freeze tissues in liquid nitrogen, and immediately transfer to –70°C. Do not allow tissues to thaw during weighing or handling prior to disruption in Buffer RLT Plus. Homogenized tissue lysates from step 2 can also be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 3. Avoid prolonged incubation, which may compromise RNA integrity.
- Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively, add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh, or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.
- When processing less than about 2 µg tissue, carrier RNA may be added to the lysate before homogenization (see "Carrier RNA", page 17). Before using for the first time, dissolve the carrier RNA (310 µg) in 1 ml RNase-free water. Store this stock solution at –20°C, and use it to make fresh dilutions for each set of preps. The concentration of this stock solution is 310 µg/ml (i.e., 310 ng/µl). To make a working solution (4 ng/µl) for 10

preps, add 5 µl stock solution to 34 µl Buffer RLT Plus and mix by pipetting. Add 6 µl of this diluted solution to 54 µl Buffer RLT Plus to give a working solution of 4 ng/µl. Add 5 µl of this solution to the lysate in step 2. **Do not add the carrier RNA to the lysate if purifying RNA for use in oligo-dT–based amplification.**

- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Before using the kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) and 6 ml RNase-free water (supplied). The procedure also requires 70% ethanol, which can be prepared by diluting ethanol (96–100%) with distilled water (not supplied).
- Buffer RLT Plus may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. **Excise the tissue sample from the animal or remove it from storage. Determine the amount of tissue. Do not use more than 5 mg. Proceed immediately to step 2.**

   Weighing tissue is the most accurate way to determine the amount. If necessary, cut the tissue on a clean surface and weigh the piece to be used.

   For RNA*later* or Allprotect stabilized tissues: Remove the tissue from the stabilization reagent using forceps and be sure to remove any crystals that may have formed. RNA in RNA*later* or Allprotect stabilized tissues is protected during cutting and weighing of tissues at ambient temperature (15–25°C). It is not necessary to cut the tissues on ice or dry ice or in a refrigerated room. Remaining tissues can be stored in RNA*later* or Allprotect Reagent. Previously stabilized tissues can be stored at –80°C without the reagent.

   For unstabilized fresh or frozen tissues: RNA in harvested tissues is not protected until the tissues are treated with RNA*later* or Allprotect Reagent, flash-frozen, or disrupted and homogenized in step 2. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible. Remaining fresh tissues can be placed into RNA*later* Reagent to stabilize RNA or in Allprotect Tissue Reagent to stabilize DNA, RNA, and protein. However, previously frozen tissues thaw too slowly in the reagent, preventing the reagent from diffusing into the tissues quickly enough to prevent RNA degradation.

2. Disrupt the tissue and homogenize the lysate in Buffer RLT Plus (do not use more than 5 mg tissue) according to step 2a, 2b, or 2c.

See "Disrupting and homogenizing starting material", page 15, for more details on disruption and homogenization.

Note: Ensure that β-ME (or DTT) is added to Buffer RLT Plus before use (see "Things to do before starting").

Note: If processing <2 µg tissue, 20 ng carrier RNA (5 µl of a 4 ng/µl solution) may be added to the lysate before homogenization. Prepare the carrier RNA as described in "Things to do before starting".

After storage in RNA*later* or Allprotect Reagent, tissues may become slightly harder than fresh or thawed tissues. Disruption and homogenization using standard methods is usually not a problem.

Note: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the AllPrep and RNeasy MinElute spin columns. Homogenization with the TissueRuptor or TissueLyser generally results in higher nucleic acid yields than with other methods. However, prolonged homogenization with these homogenizers results in greater DNA fragmentation.

2a. Disruption and homogenization using the TissueRuptor:

- **Place the tissue in a suitably sized vessel. Add 350 µl Buffer RLT Plus.**

  Note: Use a suitably sized vessel with sufficient extra headspace to accommodate foaming, which may occur during homogenization.

  Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.

- **Place the tip of the disposable probe into the vessel and operate the TissueRuptor at full speed until the lysate is homogeneous (usually 30 s). Proceed to step 3.**

  Note: To avoid damage to the TissueRuptor and disposable probe during operation, make sure the tip of the probe remains submerged in the buffer.

  Foaming may occur during homogenization. If this happens, let the homogenate stand at room temperature for 2–3 min until the foam subsides before continuing with the procedure.

2b. Disruption and homogenization using the TissueLyser:

- **Place the tissues in 2 ml microcentrifuge tubes containing one stainless steel bead (5 mm mean diameter).**

  If handling fresh or frozen tissue samples, keep the tubes on dry ice.

- **Place the tubes at room temperature. Immediately add 350 µl Buffer RLT Plus per tube.**
- **Place the tubes in the TissueLyser Adapter Set 2 x 24.**
- **Operate the TissueLyser for 2 min at 20 Hz.**

  The time depends on the tissue being processed and can be extended until the tissue is completely homogenized.
- **Rearrange the collection tubes so that the outermost tubes are innermost and the innermost tubes are outermost. Operate the TissueLyser for another 2 min at 20 Hz.**

  Rearranging the tubes allows even homogenization.
- **Carefully pipet the lysates into new microcentrifuge tubes (not supplied). Proceed to step 3.**

  Do not reuse the stainless steel beads.

2c. Disruption using a mortar and pestle followed by homogenization using a QIAshredder homogenizer or a needle and syringe:

- **Immediately place the weighed tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle.**
- **Decant tissue powder and liquid nitrogen into an RNase-free, liquid-nitrogen–cooled, 2 ml microcentrifuge tube (not supplied). Allow the liquid nitrogen to evaporate, but do not allow the tissue to thaw.**
- **Add 350 µl Buffer RLT Plus.**
- **Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed. Alternatively, pass the lysate at least 5 times through a blunt 20-gauge needle fitted to an RNase-free syringe. Proceed to step 3.**

3. Centrifuge the lysate for 3 min at full speed. Carefully remove the supernatant by pipetting, and transfer it to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm).

In some preparations, very small amounts of insoluble material will be present after the 3-min centrifugation, making the pellet invisible.

Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

4. Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 12–15. Use the flow-through for RNA purification in steps 5–11.

Note: Do not store the AllPrep DNA spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA purification

5. **Add 1 volume (usually 350 µl) of 70% ethanol to the flow-through from step 4, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 6.**

   Note: The volume of 70% ethanol to add may be less than 350 µl if some lysate was lost during homogenization and DNA isolation.

   Note: Precipitates may be visible after addition of ethanol, but this does not affect the procedure.

   Note: For maximum RNA yields from liver, use 50% ethanol instead of 70% ethanol.

6. **Transfer the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flowthrough.***

   Optional: If recovery of protein is desired, keep the flow-through on ice and follow steps E1–E5 in Appendix E on page 53.

   Reuse the collection tube in step 7.

7. **Add 700 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.***

   Reuse the collection tube in step 8.

   Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

   Optional: If purifying RNA from tissues with high DNA content and if the RNA will be used in sensitive downstream applications, we recommend performing DNase digestion by following steps F1–F4 (Appendix F, page 54) instead of step 7.

8. **Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

   Reuse the collection tube in step 9.

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

9. **Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.**

   Prepare the 80% ethanol with ethanol (96–100%) and the RNase-free water supplied with the kit.

   Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

10. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.**

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

11. **Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**

    As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.

    The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

    For RT-PCR and real-time RT-PCR with the purified RNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit www.qiagen.com/PCR . For whole transcriptome amplification (WTA) of limited amounts of RNA, we recommend the QuantiTect Whole Transcriptome Kit. For details, visit www.qiagen.com/goto/WTA .

Genomic DNA purification

12. Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 4. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (10,000 rpm). Discard the flow-through.*

Reuse the spin column in step 13.

Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

13. Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and centrifuge for 2 min at full speed to wash the spin column membrane.

Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.

Note: After centrifugation, carefully remove the AllPrep DNA spin column from the collection tube. If the column contacts the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

14. Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 50 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.

15. Repeat step 14 to elute further DNA.

To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 14.

Note: To achieve a higher DNA concentration, elute with 2 x 30 µl Buffer EB. The final DNA yield, however, may be reduced.

For PCR and real-time PCR with the purified DNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit www.qiagen.com/PCR . QIAGEN kits and services for whole genome amplification (WGA) of limited amounts of DNA are also available. For details, visit www.qiagen.com/WGA .

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

Protocol: Simultaneous Purification of Genomic DNA and Total RNA from Microdissected Cryosections

This protocol is for the purification of genomic DNA and total RNA from frozen, microdissected samples of animal and human tissues. For microdissected, formalin-fixed samples, we recommend purifying RNA with the RNeasy FFPE Kit.

Laser-microdissected tissue specimens present a particular challenge for molecular analysis, as nucleic acids must be purified from very small amounts of starting material. In addition, fixation and staining steps may compromise the integrity of RNA, and it may be necessary either to modify fixation protocols or to use cryosections from flash-frozen specimens to minimize this problem.

A wide range of equipment and consumables for sectioning, staining, and microdissection of specimens is available from Leica (www.leica-microsystems.com) and P.A.L.M. Microlaser Technologies (www.palm-mikrolaser.com).

Important points before starting

- If using the AllPrep DNA/RNA Micro Kit for the first time, read "Important Notes" (page 12).
- If preparing RNA for the first time, read Appendix A (page 44).
- To minimize RNA degradation, avoid prolonged storage of unstabilized samples at room temperature. RNA in tissues is not protected before flash-freezing in liquid nitrogen.
- Tissue lysates from step 3 can be stored at –70°C for several months. Incubate frozen lysates at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Avoid prolonged incubation, which may compromise RNA integrity.
- Buffer RLT Plus, Buffer RW1, and Buffer AW1 contain a guanidine salt and are therefore not compatible with disinfecting reagents containing bleach. See page 7 for safety information.
- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT Plus before use. Add 10 µl β-ME per 1 ml Buffer RLT Plus. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT Plus containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Alternatively,

add 20 µl of 2 M dithiothreitol (DTT) per 1 ml Buffer RLT Plus. The stock solution of 2 M DTT in water should be prepared fresh, or frozen in single-use aliquots. Buffer RLT Plus containing DTT can be stored at room temperature for up to 1 month.

- When processing <500 cells, carrier RNA may be added to the lysate before homogenization (see "Carrier RNA", page 17). Before using for the first time, dissolve the carrier RNA (310 µg) in 1 ml RNase-free water. Store this stock solution at –20°C, and use it to make fresh dilutions for each set of preps. The concentration of this stock solution is 310 µg/ml (i.e., 310 ng/µl). To make a working solution (4 ng/µl) for 10 preps, add 5 µl stock solution to 34 µl Buffer RLT Plus and mix by pipetting. Add 6 µl of this diluted solution to 54 µl Buffer RLT Plus to give a working solution of 4 ng/µl. Add 5 µl of this solution to the lysate in step 2. **Do not add the carrier RNA to the lysate if purifying RNA for use in oligo-dT–based amplification.**
- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Before using the kit for the first time, prepare 80% ethanol by mixing 24 ml ethanol (96–100%) and 6 ml RNase-free water (supplied). The procedure also requires 70% ethanol, which can be prepared by diluting ethanol (96–100%) with distilled water (not supplied).
- Buffer RLT Plus may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Procedure

Sample disruption and homogenization

1. **Collect the sample directly into an appropriate volume of Buffer RLT Plus (the volume depends on the collection vessel used for the microdissection, but should not be greater than 65 µl [Leica® instruments] or 300 µl [other instruments]).**

   Note: Ensure that β-ME (or DTT) is added to Buffer RLT Plus before use (see "Things to do before starting").

2. **If necessary, transfer the sample and buffer to a larger vessel (e.g., 1.5 or 2 ml tube). Adjust the volume to 350 µl with Buffer RLT Plus.**

   Note: If processing <500 cells, 20 ng carrier RNA (5 µl of a 4 ng/µl solution) may be added to the lysate before homogenization. Prepare the carrier RNA as described in "Things to do before starting".

3. **Vortex the sample for 30 s.**

   No further homogenization is necessary.

4. **Transfer the sample to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm).**

   Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

5. **Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25°C) or at 4°C for later DNA purification in steps 13–16. Use the flow-through for RNA purification in steps 6–12.**

   Note: Do not store the AllPrep DNA spin column at room temperature or at 4°C for long periods. Do not freeze the column.

Total RNA purification

6. **Add 1 volume (usually 350 µl) of 70% ethanol to the flow-through from step 5, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.**

   Note: The volume of 70% ethanol to add may be less than 350 µl if some lysate was lost during homogenization and DNA isolation.

   Note: Precipitates may be visible after addition of ethanol, but this does not affect the procedure.

7. **Transfer the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flowthrough.***

   Reuse the collection tube in step 8.

8. **Add 700 µl Buffer RW1 to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.***

   Reuse the collection tube in step 9.

   Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.

* Flow-through contains Buffer RLT Plus or Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

9. **Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.**

   Reuse the collection tube in step 10.

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

10. **Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.**

    Prepare the 80% ethanol with ethanol (96–100%) and the RNase-free water supplied with the kit.

    Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

11. **Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.**

    To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

    It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

12. **Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.**

    As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.

    The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

    For RT-PCR and real-time RT-PCR with the purified RNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit www.qiagen.com/PCR . For whole transcriptome amplification (WTA) of limited amounts of RNA, we recommend the QuantiTect Whole Transcriptome Kit. For details, visit www.qiagen.com/goto/WTA .

Genomic DNA purification

13. **Add 500 µl Buffer AW1 to the AllPrep DNA spin column from step 5. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (10,000 rpm). Discard the flow-through.***

    Reuse the spin column in step 14.

    Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW1 before use (see "Things to do before starting").

14. **Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and centrifuge for 2 min at full speed to wash the spin column membrane.**

    Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use (see "Things to do before starting").

    The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with downstream reactions.

    Note: After centrifugation, carefully remove the AllPrep DNA spin column from the collection tube. If the column contacts the flow-through, empty the collection tube and centrifuge the spin column again for 1 min at full speed.

15. **Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 50 µl Buffer EB (preheated to 70°C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at ≥8000 x *g* (10,000 rpm) to elute the DNA.**

16. **Repeat step 15 to elute further DNA.**

    To prevent dilution of the first DNA eluate, use a new 1.5 ml collection tube (not supplied) to collect the second DNA eluate. To combine the first and second DNA eluates, reuse the collection tube from step 15.

    Note: To achieve a higher DNA concentration, elute with 2 x 30 µl Buffer EB. The final DNA yield, however, may be reduced.

    For PCR and real-time PCR with the purified DNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. For details, visit www.qiagen.com/PCR . QIAGEN kits and services for whole genome amplification (WGA) of limited amounts of DNA are also available. For details, visit www.qiagen.com/WGA .

* Flow-through contains Buffer AW1 and is therefore not compatible with bleach. See page 7 for safety information.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or molecular biology applications (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Clogged AllPrep DNA or RNeasy MinElute spin column	
a) Inefficient disruption and/or homogenization	See "Disrupting and homogenizing starting material" (page 15) for details on disruption and homogenization methods. Increase *g*-force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material (see the individual protocols) and/or increase the homogenization time.
b) Too much starting material	Reduce the amount of starting material. It is essential to use the correct amount of starting material (see page 12).
c) Centrifugation temperature too low	The centrifugation temperature should be 20–25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the spin column. If this happens, set the centrifugation temperature to 25°C. Warm the lysate to 37°C before transferring it to the AllPrep DNA spin column.
Low nucleic acid yield	
a) Insufficient disruption and homogenization	See "Disrupting and homogenizing starting material" (page 15) for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material (see the individual protocols) and/or increase the volume of lysis buffer and the homogenization time.
b) Too much starting material	Overloading the spin columns significantly reduces nucleic acid yields. Reduce the amount of starting material (see the individual protocols).

	Comments and suggestions
c) RNA still bound to RNeasy MinElute spin column membrane	Repeat RNA elution, but incubate the RNeasy MinElute spin column on the benchtop for 10 min with RNase-free water before centrifuging.
d) DNA still bound to AllPrep DNA spin column membrane	Repeat DNA elution, but incubate the AllPrep DNA spin column on the benchtop for 10 min with Buffer EB before centrifuging.
e) Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the RNeasy MinElute spin column membrane. After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.
f) Incomplete removal of cell-culture medium (cell samples)	When processing cultured cells, ensure complete removal of cell-culture medium after harvesting cells (see protocol, page 19).

DNA contaminated with RNA

a) Lysate applied to the AllPrep DNA spin column contains ethanol	Add ethanol to the lysate after passing the lysate through the AllPrep DNA spin column.
b) Sample is affecting pH of homogenate	The final homogenate should have a pH of 7. Make sure that the sample is not highly acidic or basic.

Contamination of RNA with DNA affects downstream applications

a) Cell number too high	For some cell types, the efficiency of DNA binding to the AllPrep DNA spin column may be reduced when processing very high cell numbers. If the eluted RNA contains substantial DNA contamination, try processing smaller cell numbers.

	Comments and suggestions
b) Incomplete removal of cell-culture medium or stabilization reagent	Be sure to remove any excess cell-culture medium or stabilization reagent to prevent significant dilution of the lysis buffer. The AllPrep DNA spin column will not bind DNA effectively if the lysis buffer is diluted.
c) Tissue has high DNA content	For certain tissues with extremely high DNA content (e.g., thymus), trace amounts of DNA may pass through the AllPrep DNA spin column. Try using smaller samples. Alternatively, perform DNase digestion on the RNeasy MinElute spin column membrane (see Appendix F, page 54).
Low A_{260}/A_{280} value in RNA eluate	
Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris·Cl,* pH 7.5, not RNase-free water, to dilute the sample before measuring purity (see Appendix B, page 46).
RNA degraded	
a) Inappropriate handling of starting material	Ensure that tissue samples are properly stabilized and stored in RNA*later* RNA Stabilization Reagent or Allprotect Tissue Reagent. For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the AllPrep DNA/RNA procedure quickly, especially the first few steps. See Appendix A (page 44) and "Handling and storing starting material" (page 14).
b) RNase contamination	Although all AllPrep buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the AllPrep DNA/RNA procedure or later handling. See Appendix A (page 44) for general remarks on handling RNA.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

	Comments and suggestions
DNA fragmented	
Homogenization too vigorous	The length of the purified DNA (usually 15–30 kb) depends strongly on the homogenization conditions. If longer DNA fragments are required, keep the homogenization time to a minimum or use a gentler homogenization method if possible (e.g., use a QIAshredder homogenizer instead of a rotor–stator homogenizer).
Nucleic acid concentration too low	
Elution volume too high	Elute nucleic acids in a smaller volume. Do not use less than 30 µl Buffer EB for the AllPrep DNA spin column, or less than 12 µl RNase-free water for the RNeasy MinElute spin column. Although eluting in smaller volumes results in increased nucleic acid concentrations, yields may be reduced.
Nucleic acids do not perform well in downstream experiments	
a) Salt carryover during elution	Ensure that buffers are at 20–30°C. Ensure that the correct buffer is used for each step of the procedure. When reusing collection tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels.
b) Ethanol carryover	After the wash with 80% ethanol, be sure to centrifuge at full speed for 5 min to dry the RNeasy MinElute spin column membrane. After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

Appendix A: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 45). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,* thoroughly rinsed, and oven baked at 240°C for at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allowed to dry.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: AllPrep buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Appendix B: Storage, Quantification, and Determination of Quality of RNA

Storage of RNA

Purified RNA may be stored at –20°C or –70°C in RNase-free water. Under these conditions, no degradation of RNA is detectable after 1 year.

Quantification of RNA

The concentration of RNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of RNA" below). For small amounts of RNA, however, it may not be possible to accurately determine amounts photometrically. Small amounts of RNA can be quantified using an Agilent® 2100 bioanalyzer, fluorometric quantification, or quantitative, real-time RT-PCR. When purifying RNA from particularly small samples (e.g., laser-microdissected samples), quantitative, real-time RT-PCR should be used for quantification.

Spectrophotometric quantification of RNA

To ensure significance, A_{260} readings should be greater than 0.15. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml ($A_{260}=1 \rightarrow 44$ µg/ml). This relation is valid only for measurements at a neutral pH. Therefore, if it is necessary to dilute the RNA sample, this should be done in a buffer with neutral pH.* As discussed below (see "Purity of RNA", page 47), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity.

When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be accomplished by washing cuvettes with 0.1 M NaOH, 1 mM EDTA,* followed by washing with RNase-free water (see "Solutions", page 45). Use the buffer in which the RNA is diluted to zero the spectrophotometer. An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 100 µl

Dilution = 10 µl of RNA sample + 490 µl of 10 mM Tris·Cl,* pH 7.0 (1/50 dilution)

Measure absorbance of diluted sample in a 1 ml cuvette (RNase-free)

A_{260} = 0.2

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Concentration of RNA sample	= 44 µg/ml x A_{260} x dilution factor
	= 44 µg/ml x 0.2 x 50
	= 440 µg/ml
Total amount	= concentration x volume in milliliters
	= 440 µg/ml x 0.1 ml
	= 44 µg of RNA

Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A_{260}/A_{280}) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV spectrum, such as protein. However, the A_{260}/A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/A_{280} ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris·Cl, pH 7.5. Pure RNA has an A_{260}/A_{280} ratio of 1.9–2.1† in 10 mM Tris·Cl, pH 7.5. Always be sure to calibrate the spectrophotometer with the same solution used for dilution.

For determination of RNA concentration, however, we recommend dilution of the sample in a buffer with neutral pH since the relationship between absorbance and concentration (A_{260} reading of 1 = 44 µg/ml RNA) is based on an extinction coefficient calculated for RNA at neutral pH (see "Spectrophotometric quantification of RNA", page 46).

DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. While the the vast majority of cellular DNA will bind to the AllPrep DNA spin column, trace amounts may still remain in the purified RNA, depending on the amount and nature of the sample.

For analysis of very low abundance targets, any interference by residual DNA contamination can be detected by performing real-time RT-PCR control experiments in which no reverse transcriptase is added prior to the PCR step.

To prevent any interference by DNA in real-time RT-PCR applications, such as with Applied Biosystems® and LightCycler® instruments, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. QuantiTect Primer Assays from QIAGEN are designed for

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† Values up to 2.3 are routinely obtained for pure RNA (in 10 mM Tris·Cl, pH 7.5) with some spectrophotometers.

SYBR® Green based real-time RT-PCR analysis of RNA sequences (without detection of genomic DNA) where possible. For real-time RT-PCR assays where amplification of genomic DNA cannot be avoided, the QuantiTect Reverse Transcription Kit provides fast cDNA synthesis with integrated removal of genomic DNA contamination (see ordering information, page 56).

Integrity of RNA

The integrity and size distribution of total RNA purified with the AllPrep DNA/RNA Micro Kit can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining* or by using an Agilent 2100 bioanalyzer. The respective ribosomal RNAs should appear as sharp bands or peaks. The apparent ratio of 28S rRNA to 18S RNA should be approximately 2:1. If the ribosomal bands or peaks of a specific sample are not sharp, but appear as a smear towards smaller sized RNAs, it is likely that the sample suffered major degradation either before or during RNA purification.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Appendix C: Storage, Quantification, and Determination of Quality of Genomic DNA

Storage of DNA

For long-term storage, purified DNA in Buffer EB can be stored at –20°C. Avoid any contamination, as this may lead to DNA degradation. We recommend storing samples in aliquots in order to avoid repeated freezing and thawing.

Quantification of DNA

The concentration of DNA can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (see "Spectrophotometric quantification of DNA" below). For small amounts of DNA, however, it may not be possible to accurately determine amounts photometrically. Small amounts of DNA can be quantified using quantitative, real-time PCR or fluorometric quantification. When purifying DNA from particularly small samples (e.g., laser-microdissected samples), quantitative, real-time PCR should be used for quantification.

Spectrophotometric quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer using a quartz cuvette. For greatest accuracy, readings should be between 0.1 and 1.0. Using a standard 1 cm path length, an absorbance of 1 unit at 260 nm corresponds to 50 µg genomic DNA per ml (A_{260} =1 → 50 µg/ml). This relation is valid only for measurements made at neutral pH. Therefore, samples should be diluted in a low-salt buffer with neutral pH (e.g., Tris·Cl, pH 7.0).* Use the buffer in which the DNA is diluted to zero the spectrophotometer. An example of the calculation involved in DNA quantification is shown below:

Volume of DNA sample = 100 µl

Dilution = 20 µl of DNA sample + 180 µl of buffer (1/10 dilution)

Measure absorbance of diluted sample in a 0.2 ml cuvette

A_{260} = 0.2

Concentration of DNA sample = 50 µg/ml x A_{260} x dilution factor

= 50 µg/ml x 0.2 x 10

= 100 µg/ml

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Total amount = concentration x volume of sample in milliliters

= 100 µg/ml x 0.1 ml

= 10 µg of DNA

RNA concentration can also be determined by measuring the absorbance at 260 nm. If the eluate contains both DNA and RNA, a fluorometer must be used to quantify the DNA.

Determination of DNA purity

The ratio of the readings at 260 nm and 280 nm (A_{260}/ A_{280}) provides an estimate of DNA purity with respect to contaminants that absorb UV light, such as protein. The A_{260}/ A_{280} ratio is influenced considerably by pH. Since water is not buffered, the pH and the resulting A_{260}/ A_{280} ratio can vary greatly. Lower pH results in a lower A_{260}/ A_{280} ratio and reduced sensitivity to protein contamination.* For accurate A_{260}/ A_{280} values, we recommend measuring absorbance in a slightly alkaline buffer (e.g., 10 mM Tris·Cl, pH 7.5). Make sure to zero the spectrophotometer with the appropriate buffer.

Pure DNA has an A_{260}/ A_{280} ratio of 1.7–1.9. Scanning the absorbance from 220–320 nm will show whether there are contaminants affecting absorbance at 260 nm. Absorbance scans should show a peak at 260 nm and an overall smooth shape.

Determination of DNA length

The precise length of genomic DNA can be determined by pulsed-field gel electrophoresis (PFGE) through an agarose gel. The DNA should be concentrated by alcohol† precipitation and reconstituted by gentle agitation in approximately 30 µl TE buffer, pH 8.0,† for at least 30 minutes at 60°C. Avoid drying the DNA pellet for more than 10 minutes at room temperature (15–25°C), since over-dried genomic DNA is very difficult to redissolve. Load 3–5 µg of DNA per well. Standard PFGE conditions are as follows:

- 1% agarose† gel in 0.5x TBE electrophoresis buffer†
- Switch intervals = 5–40 s
- Run time = 17 h
- Voltage = 170 V

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques **22**, 474.

† When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Appendix D: Purification of Total RNA Containing Small RNAs from Cells

The following procedure allows the purification of total RNA containing small RNAs such as miRNA from animal and human cells.

Reagents to be supplied by user

- Ethanol (100%)*

Important points before starting

- Perform all steps of the procedure at room temperature (15–25°C). During the procedure, work quickly.
- Perform all centrifugation steps at 20–25°C in a standard microcentrifuge. Ensure that the centrifuge does not cool below 20°C.

Things to do before starting

- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Procedure

Carry out the protocol starting on page 19 up to and including step 5. Instead of performing steps 6–12 (purification of total RNA >200 nucleotides), follow steps D1–D6 below (purification of total RNA containing small RNAs).

D1. Add 1.5 volumes (usually 525 µl) of 100% ethanol to the flow-through from the AllPrep DNA spin column, and mix well by pipetting. Do not centrifuge. Proceed immediately to step D2.

Note: The volume of 100% ethanol to add may be less than 525 µl if some lysate was lost during homogenization and DNA isolation.

Note: Precipitates may be visible after addition of ethanol, but this does not affect the procedure.

D2. Transfer 700 µl of the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.†

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† Flow-through contains Buffer RLT Plus and is therefore not compatible with bleach. See page 7 for safety information.

Repeat step D2 until the whole sample has passed through the membrane. Discard the flow-through each time.

Reuse the collection tube in step D3.

D3. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

Reuse the collection tube in step D4.

Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

D4. Add 500 µl Buffer RPE to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the collection tube with the flow-through.

Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

D5. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied). Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the collection tube with the flow-through.

To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

It is important to dry the spin column membrane, since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

D6. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied). Add 14 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA (total RNA containing small RNAs).

As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%. Do not elute with less than 10 µl RNase-free water, as the spin column membrane will not be sufficiently hydrated.

The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

For real-time RT-PCR with the purified RNA, QIAGEN offers the miScript System, which allows detection of hundreds of miRNAs from a single cDNA synthesis reaction. For details, visit www.qiagen.com/miRNA .

Appendix E: Acetone Precipitation of Protein from Lysates

The following procedure describes how to recover denatured protein by acetone precipitation from lysates of cells and easy-to-lyse tissues.

Reagents to be supplied by user

- Ice
- Acetone*
- Optional: Ethanol*
- Buffer* for downstream application (e.g., loading buffer for SDS-PAGE gel)

Important points before starting

- **Do not use trichloroacetic acid (TCA) to precipitate protein from Buffer RLT Plus lysates.** This buffer contain guanidine thiocyanate, which can form highly reactive compounds when combined with acidic solutions.

Procedure

Bind total RNA to the RNeasy MinElute spin column as described in the cell protocol (from page 19, steps 1–7) or the tissue protocol (from page 27, steps 1–6). Then follow steps E1–E5 below to precipitate protein.

E1. Add 4 volumes of ice-cold acetone to the flow-through from the RNeasy MinElute spin column.

E2. Incubate for 30 min on ice or at –20°C.

E3. Centrifuge for 10 min at full speed in a benchtop centrifuge. Discard the supernatant and air-dry the pellet.†

E4. Optional: Wash the pellet with 100 µl ice-cold ethanol and air-dry.

Do not overdry the pellet as this may make resuspension more difficult.

E5. Resuspend the pellet in the buffer for your downstream application.

Sodium dodecyl sulfate (SDS) causes guanidine salts to precipitate. In case the pellet contains traces of guanidine thiocyanate, load the sample onto an SDS-PAGE gel immediately after heating for 7 minutes at 95°C.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† Supernatant contains guanidine thiocyanate and is therefore not compatible with bleach. See page 7 for safety information.

Appendix F: Optional On-Column DNase Digestion Using the RNase-Free DNase Set

Although DNA binds very efficiently to the AllPrep DNA spin column, some tissues contain very high amounts of DNA (e.g., spleen and thymus) that may result in trace amounts of DNA passing through the membrane. For these tissues, DNase digestion can be performed on the RNeasy MinElute spin column membrane if the eluted RNA will be used in downstream applications sensitive to very small amounts of DNA. **Tissues containing moderate amounts of DNA and cultured cells do not require DNase digestion.**

The QIAGEN RNase-Free DNase Set (see page 56 for ordering information) provides efficient on-column digestion of DNA during RNA purification. The DNase is efficiently removed in subsequent wash steps.

Note: Buffer RDD supplied with the QIAGEN RNase-Free DNase Set is specially optimized for on-column DNase digestion. Use of other DNase buffers may affect the binding of the RNA to the RNeasy MinElute spin column membrane, reducing RNA yield and integrity.

Preparation of tissue homogenates and binding of RNA to the RNeasy MinElute spin column membrane are performed according to the protocol starting on page 27. After washing with a reduced volume of Buffer RW1, RNA is treated with DNase I while bound to the spin column membrane. DNase I is removed by a second wash with Buffer RW1. Washing with Buffer RPE and elution are then performed according to the protocol on page 27.

Important points before starting

- **Do not vortex the reconstituted DNase I.** DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the vial.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. **Do not vortex.**
- For long-term storage of DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

Carry out the protocol starting on page 27 up to and including step 6. Instead of peforming step 7 (the wash with Buffer RW1), follow steps F1–F4 below.

F1. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.*

Reuse the collection tube in step F4.

F2. Add 10 µl DNase I stock solution (see above) to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

Buffer RDD is supplied with the RNase-Free DNase Set.

Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

F3. Add the DNase I incubation mix (80 µl) directly to the RNeasy MinElute spin column membrane, and place on the benchtop (20–30°C) for 15 min.

Note: Be sure to add the DNase I incubation mix directly to the RNeasy spin column membrane. DNase digestion will be incomplete if part of the mix sticks to the walls or the O-ring of the spin column.

F4. Add 350 µl Buffer RW1 to the RNeasy MinElute spin column, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.* Continue with step 8 of the protocol on page 27 (i.e., the wash with Buffer RPE).

Reuse the collection tube in step 8.

* Flow-through contains Buffer RW1 and is therefore not compatible with bleach. See page 7 for safety information.

Ordering Information

Product	Contents	Cat. no.
AllPrep DNA/RNA Micro Kit (50)	50 AllPrep DNA Mini Spin Columns, 50 RNeasy MinElute Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80284
AllPrep DNA/RNA Mini Kit (50)*	50 AllPrep DNA Mini Spin Columns, 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80204
Accessories		
RNAprotect Cell Reagent (250 ml)	250 ml RNAprotect Cell Reagent	76526
RNA*later* RNA Stabilization Reagent (50 ml)	For stabilization of RNA in 25 x 200 mg tissue samples: 50 ml RNA*later* RNA Stabilization Reagent	76104
RNA*later* RNA Stabilization Reagent (250 ml)	For stabilization of RNA in 125 x 200 mg tissue samples: 250 ml RNA*later* RNA Stabilization Reagent	76106
RNA*later* TissueProtect Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA*later* RNA Stabilization Reagent each	76154
RNA*later* TissueProtect Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNA*later* RNA Stabilization Reagent each	76163
Allprotect Tissue Reagent (100 ml)	For stabilization of DNA/RNA/protein in 50 x 200 mg tissue samples: 100 ml Allprotect Tissue Reagent, Allprotect Reagent Pump	76405
RNase-Free DNase Set (50)	For 50 RNA minipreps: DNase I, Buffer RDD, and Water (all RNase-Free)	79254
Collection Tubes (2 ml)	1000 x 2 ml Collection Tubes	19201

* For DNA and RNA purification from up to 1 x 10^7 cells or 30 mg tissue.

Product	Contents	Cat. no.
QIAshredder (50)	50 disposable cell-lysate homogenizers	79654
QIAshredder (250)	250 disposable cell-lysate homogenizers	79656
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	Varies*
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890
TissueLyser	Universal laboratory mixer-mill disruptor	Varies*
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser	69982
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
Related products		
AllPrep DNA/RNA/Protein Mini Kit — for simultaneous purification of DNA, RNA, and protein from cells and tissues		
AllPrep DNA/RNA/Protein Mini Kit (50)	50 AllPrep DNA Spin Columns, 50 RNeasy Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80004
AllPrep RNA/Protein Kit — for simultaneous purification of total RNA and protein from cultured cells		
AllPrep RNA/Protein Kit (50)	50 AllPrep Mini Spin Columns, 50 RNeasy Mini Spin Columns, 50 Protein Cleanup Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	80404

* Visit www.qiagen.com/automation to find out more about the TissueRuptor and TissueLyser and to order.

Product	Contents	Cat. no.
DNeasy Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)*	50 DNeasy Mini Spin Columns, Proteinase K, Buffers, Collection Tubes	69504
RNeasy Fibrous Tissue Mini Kit — for purification of total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)	50 RNeasy Mini Spin Columns, Collection Tubes, Proteinase K, RNase-Free DNase I, RNase-Free Reagents and Buffers	74704
RNeasy FFPE Kit — for purification of high yields of usable RNA from FFPE tissue sections		
RNeasy FFPE Kit (50)	50 RNeasy MinElute Spin Columns, 50 gDNA Eliminator Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74404
REPLI-g Kits — for highly uniform whole genome amplification from small or precious samples		
REPLI-g Mini Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions (typical yield 10 µg per reaction)	150023
QIAGEN Fast Cycling PCR Kit — for fast and specific PCR on any thermal cycler		
QIAGEN Fast Cycling PCR Kit (200)*	For 200 x 20 µl reactions: 2 x 1 ml QIAGEN Fast Cycling PCR Master Mix, 10x CoralLoad Fast Cycling Dye, Q-Solution, RNase-Free Water	203743
QuantiTect Whole Transcriptome Kit — for unlimited real-time PCR analysis from precious RNA samples		
QuantiTect Whole Transcriptome Kit (25)*	For 25 x 50 µl whole-transcriptome-amplification reactions: T-Script Enzyme, Ligation Enzymes, REPLI-g DNA Polymerase, Reagents and Buffers	207043

* Larger kit size and/or format available; please inquire.

Product	Contents	Cat. no.
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 µl reverse-transcription reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, RNase-Free Water	205311
QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at www.qiagen.com/GeneGlobe)		
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions or 400 x 25 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies
QuantiFast™ SYBR Green PCR Kit — for fast SYBR Green based real-time PCR and two-step RT-PCR		
QuantiFast SYBR Green PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 2 x 2 ml RNase-Free Water	204054
QuantiFast SYBR Green RT-PCR Kit — for fast SYBR Green based real-time one-step RT-PCR		
QuantiFast SYBR Green RT-PCR Kit (400)*	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix (contains ROX dye), 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204154

RNAprotect Cell Reagent, RNA*later* RNA Stabilization Reagent, RNA*later* TissueProtect Tubes, Allprotect Tissue Reagent, AllPrep Kits, the DNeasy Blood & Tissue Kit, the RNeasy Fibrous Tissue Mini Kit, the RNeasy FFPE Kit, REPLI-g Kits, the QIAGEN Fast Cycling PCR Kit, the QuantiTect Whole Transcriptome Kit, the QuantiTect Reverse Transcription Kit, QuantiTect Primer Assays, and QuantiFast Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

* Other kit sizes available; please inquire.

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